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Moderation of iodoacetate-induced experimental osteoarthritis in rats by matrix metalloproteinase inhibitors

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Summary

Objective: To determine the effect of matrix metalloproteinase (MMP) inhibitors in mono-iodoacetate-induced arthritis in rats.

Design: The ability of compounds to inhibit MMPs in vitro was assessed kinetically using a quenched fluorescent substrate. Rats were injected with iodoacetate intraarticularly in one knee joint and damage to the tibial plateau was evaluated from digitized images captured using an image analyser and by histology. Collagenase and gelatinase activity in cartilage from iodoacetate injected knees were evaluated using ^{3}H-rat type I collagen and gelatin zymography, respectively.

Results: Collagenase and gelatinase activity significantly increased in the knee cartilage of rats injected with iodoacetate with peak activity by day 7. Three MMP inhibitors were examined for their efficacy in the rat iodoacetate-induced arthritis model. Significant (P<0.05) inhibition of cartilage damage was observed in animals treated orally with 35 mg/kg b.i.d. of the three different MMP inhibitors. Inhibition of cartilage damage by the MMP inhibitors ranged from 36–42%.

Conclusion: MMP inhibitors are partially protective against cartilage and subchondral bone damage induced by iodoacetate. These results support an important role for MMPs in mediating the joint damage in this model of arthritis. © 2001 OsteoArthritis Research Society International

Key words: Matrix metalloproteinase inhibitors, Iodoacetate-induced arthritis, Collagenase, Gelatinase.

Introduction

Osteoarthritis (OA) is a degenerative disease that effects a large population and results in significant morbidity and disability. The initiating events that result in the cartilage degradation, sclerosis of subchondral bone and osteophyte formation that are characteristic of OA are poorly understood. Numerous animal models that mimic aspects of human OA have been developed as tools to study the pathophysiology of the disease and to evaluate therapeutic modalities. OA can be induced in experimental animals by a variety of insults including surgically-induced osteoarthritis in dogs[1], rabbits[2] and guinea pigs[3,4] intraarticular injection of bacterial collagenase[5] or iodoacetate[6–10]. In addition, small animal models of spontaneous OA have been described in the guinea pig[11] and mouse[12,13].

Matrix metalloproteinases (MMPs) have been implicated in the cartilage matrix degradation associated with both OA and rheumatoid arthritis (RA)[14–20]. Inhibition of MMPs has long been proposed as a therapeutic modality to prevent cartilage degradation that occurs as a result of the arthritic process[21]. Numerous inhibitors of the MMPs have been proposed as potential therapeutic agents and the various types of compounds and their activities have been reviewed[22].

Experimental models of RA and OA have been developed to study the pathophysiology of these diseases and to evaluate potential antiarthritic agents. However, there are few studies on the effect of MMP inhibitors in experimental models of arthritis. Tetracycline derivatives[23] and broad spectrum MMP inhibitors have been shown to significantly inhibit joint swelling and bone destruction in adjuvant arthritis in rats[24,25]. In one of these studies an MMP inhibitor was found to significantly inhibit cartilage degradation and pannus formation[25]. Administration of doxycycline was found to reduce the severity of cartilage lesions and the amounts of collagenolytic and gelatinolytic activity in cartilage extracts from dogs with OA induced by dorsal root ganglionectomy followed by anterior cruciate ligament transection[26]. However, doxycycline did not inhibit spontaneous OA in guinea pigs whereas, a chemically modified tetracycline (CMT-7) was effective[27]. Recently, the collagenase selective MMP inhibitor, Ro 32-3555 was shown to inhibit cartilage and bone changes that occur spontaneously in the STR/ORT mouse[28].

The use of iodoacetate to chemically induce degenerative arthritis was first described by Kalbhen in chickens and subsequently in a number of other species[6–10,29]. The severity of the cartilage lesions, suppression of mobility and inhibition of proteoglycan synthesis was directly related to the dose of iodoacetate[30]. The rapid development of joint pathology in a manner that can be controlled by the dose of

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iodoacetate injected provides a useful model system to evaluate potential modulators of OA. In the present study we demonstrate the up-regulation of MMPs in knee cartilage of iodoacetate-injected rats and the inhibition of degenerative changes by the administration of MMP inhibitors, thereby providing the first evidence that matrix metalloproteinases are important mediators of the joint pathology in this model.

Materials and methods

ANIMALS

Sprague-Dawley male rats weighing 220–230 g (Harlan, Indianapolis, IN) were housed singly in wire cages in sanitary ventilated animal rooms with controlled temperature, humidity and regular light cycles. Rodent chow (Ralston-Purina, Richmond, IN) and water were allowed *ad libitum*. Animals were acclimated for one week before use.

It is the policy of Procter & Gamble Pharmaceuticals that all animals be housed, fed, and handled in compliance with the standards set forth by the Animal Welfare Act as amended. Where standards are not indicated in the Animal Welfare Act the recommendations on HHS Publications (NIH) No. 85-23, Guide for the Care and Use of Laboratory Animals were followed. The personnel at Procter & Gamble Pharmaceuticals adhered to and were in compliance with the above stated policy.

INDUCTION OF IODOACETATE-INDUCED ARTHRITIS

Arthritis was induced by a single intraarticular injection of iodoacetate into the knee joint of rats anesthetized using (3:1) CO_2/O_2. A 10 mg/ml concentration of monosodium iodoacetate (MIA) (Aldrich Chemical, Milwaukee, WI) was prepared using injectable saline as the vehicle. After appropriate anesthesia each rat was positioned on their back and the left leg was flexed 90° at the knee. The patellar ligament was palpated below the patella and the injection was made into this region. Each rat received 0.025 ml intraarticular injection into the left knee using a glass gas tight syringe with a 27 gauge 0.5 inch needle. Care was taken not to advance the needle too far into the cruciate ligaments.

TREATMENTS

Animals were dosed orally with MMP inhibitor or vehicle twice daily at 12 h intervals (b.i.d.) either for the first 7 days after iodoacetate injection or for 21 days. Experiments described below demonstrated that peak MMP levels were between days 3 and 7 following iodoacetate injection. Accordingly, a 7-day dosing protocol was developed. Data from these experiments confirmed that dosing with a MMP inhibitor for 7 days was almost as efficacious as administering compound for the duration of the 21-day study. Therefore, in experiments where compounds were screened at a single dose, a 7-day dosing protocol was used to reduce the amount of MMP inhibitor required, whereas, dose response experiments were performed using 21-day dosing. Vehicle control and MMP inhibitor treated groups consisted of 15 animals each. Animals were sacrificed 21 days after iodoacetate injection and the left joint was immediately disarticulated and fixed in 10% buffered formalin for 24–48 h prior to capturing the image. There were no deaths or observable toxic effects in any of the animals dosed with vehicle or MMPIs.

ARTICULAR CARTILAGE LESION SCORE

After fixation, an image of the tibial cartilage was captured using an Optimas image analysis system (Optimas, Media Cybernetics LP, Silver Spring, MA). The tibial plateau was utilized for image analysis because it provided a relatively flat surface compared with the femoral condyles, allowing the image analysis camera to focus on the entire cartilage surface. Three independent observers assessed cartilage damage in a blinded manner using a scale of 0–4 of increasing severity (0=normal; 4=maximum severity) (Fig. 1). The cross observer correlation was 0.83 (Pearson's correlation coefficient). This scoring system was similar to that described previously by Guincamp *et al*.[30].

HISTOLOGY

Knee joints were placed in 10% buffered formalin for 24 h followed by decalcification in 20% EDTA. The progression of the decalcification was assessed by X-ray and required approximately 14–21 days. The fixed, decalcified tissue was embedded in hard paraffin wax and was processed using conventional dehydrating and clearing agents and 7 μM sections were cut. Sections were stained with safranin O-fast green.

The effect of MMP inhibition on joint damage was assessed histologically by evaluating the effect of compound on the subchondral bone lesions that were characterized by an infiltration by vascularized fibrous tissue and mononuclear cells. Since the subchondral bone lesions that occur in the rat iodoacetate model are widespread and random, multiple sections were evaluated for each animal. The tibial surface was cut coronally across both tibial plateaus and the two halves were embedded into the same paraffin block. Ten step sections 150 μm apart were made in each block providing a total of 20 samples representing the entire tibial plateau. The mean damage score from the 20 samples from each animal (15 animals/group) was used to determine the mean±S.E.M. for each treatment group. Subchondral bone lesions were assessed on a scale of 0–4 where 0=no subchondral lesions with cellular infiltration, 1=minimal subchondral lesions (1–2) [for an example see Fig. 3(f)], <5% of the tibial plateau involved, 2=mild subchondral lesions (2–3), <15% of the tibial plateau involved, 3=moderate subchondral lesions (4–5), <25% of the tibial plateau involved and 4=severe subchondral lesions (5 or more), >25% of the tibial plateau involved.

Proteoglycan content was scored in safranin O/fast green stained slides on a scale of 0–4 where 0=no loss of proteoglycan staining relative to a normal control, 1=minimal loss, 2=mild loss, 3=moderate loss and 4=total loss of proteoglycan staining. A single observer performed the histological assessment in a blinded manner.

STATISTICAL ANALYSIS

Data were analysed using a non-parametric procedure (Wilcoxon rank sum). The data are expressed as the mean±S.E.M. and statistical differences from the vehicle treated control ($P<0.05$) are denoted with an asterisk.

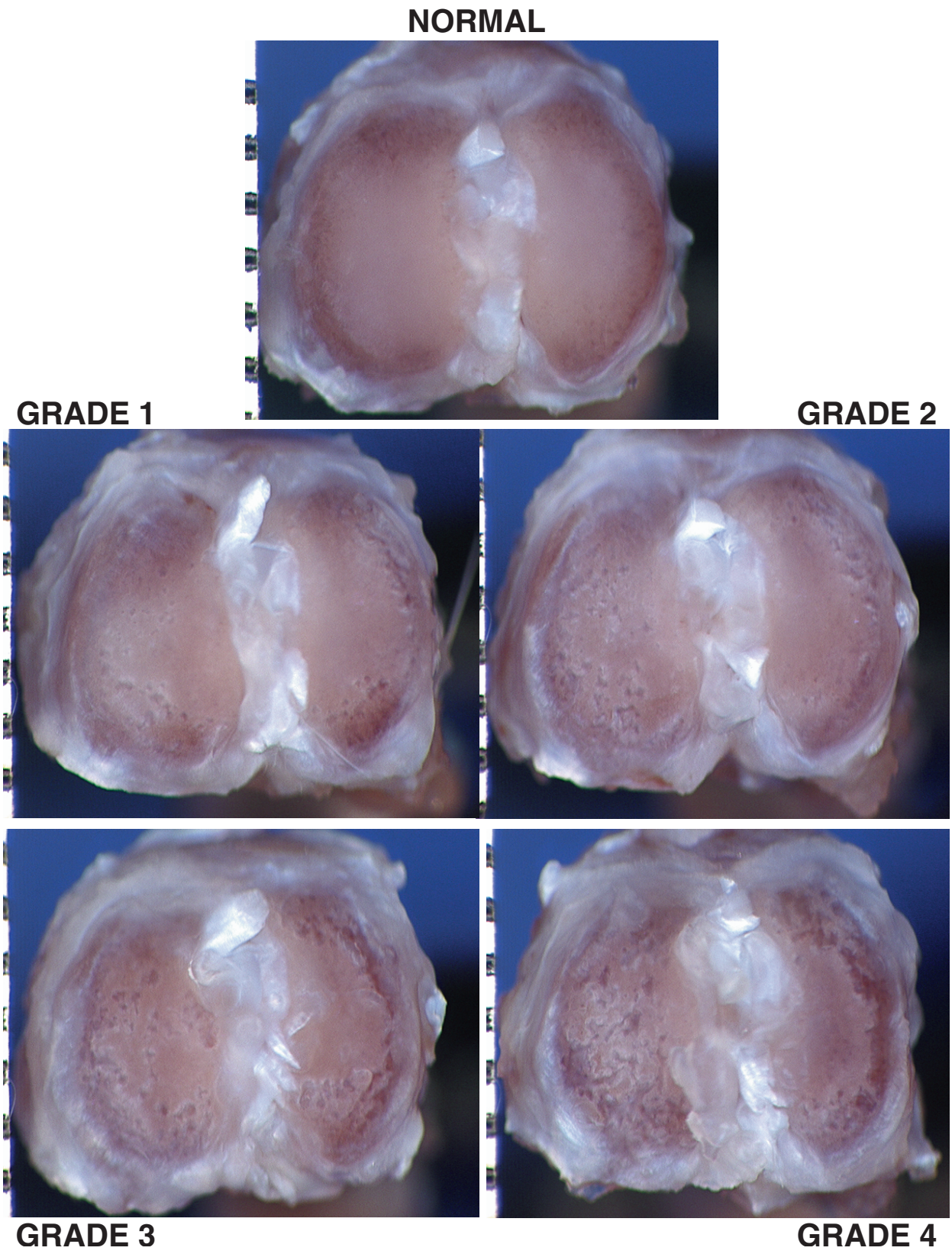


Fig. 1. Macroscopic scoring of cartilage lesions. Cartilage damage was assessed from images captured and magnified using an image analyser by three independent observers in a blinded manner using a scale of 0–4 of increasing severity (0=normal; 4=maximum severity).

COLLECTION AND PREPARATION OF CARTILAGE EXTRACTS

The hind limbs from rats were collected and frozen at −70°C until processed. After thawing, the tissue surrounding the joint was removed, the joint was disarticulated and the cartilage scraped from the surface of the tibia and femur using a no. 10 scalpel blade (Bard-Parker, Becton Dickinson, Franklin Lakes, NJ) and an illuminated magnifier. The cartilage chips were placed into a pre-weighed vial, the weight of the cartilage was determined and cold extractant was added to the vial.

Collagenase was extracted using a two step procedure. 10 μl of 0.25% Triton X-100 with 0.01 M $CaCl_2$ was added per mg of cartilage. Extraction was performed for 20 min at 4°C followed by centrifugation in a microfuge for 20 min at 13,000 rpm. Because the cartilage slices were so thin no homogenization was required. The supernatant was removed and the pellet extracted with 50 mM Tris/HCl, 0.1 M $CaCl_2$, 0.15 M NaCl, pH 7.5. The extract was heated for 4 min at 60°C in a water bath and centrifugation repeated as described above. The supernatant was collected and combined with the supernatant from the Triton-X 100 extract.

Extraction of the gelatinases was performed by adding 30 μl of 2% sodium dodecyl sulfate (SDS) per mg of cartilage and the mixture was allowed to stand for 1 h at 4°C. The mixture was centrifuged in a microfuge for 20 min at 13,000 rpm and the supernatant collected. This extraction and centrifugation procedure was repeated and the supernatants combined. Preliminary experiments determined that extraction of gelatinases with SDS resulted in better recovery of activity than extraction with 4 M GuHCL (J. F. Woessner, unpublished observations).

COLLAGENASE ASSAY

Tissue inhibitor of metalloproteinase (TIMP) present in cartilage extracts (50 μl) was inactivated by incubation with 10 μl of 10 mM dithiothreitol (DTT) at 37°C for 30 min to reduce disulfide bridges in TIMP. The DTT was inactivated by the addition of 10 μl of iodoacetamide. Telopeptide free rat skin ^{3}H-collagen 5.3 μ curie/mg in 50 mM Tris-HCl buffer pH, 7.5 was added to give approximately 100,000 cpm of collagen. Aminophenylmercuric acetate (APMA) was added to the reaction mixture to a final concentration of 0.25 mM and the mixture was incubated at 30°C for 48 h. As a positive control, rat collagenase was incubated with the collagen substrate. Collagenase digestion was performed in a rigorously controlled water bath (30°±0.1°C).

GELATINASE ASSAYS

Gelatinase activity was measured by zymography using the method of Hibbs *et al.*[31]. Briefly, dilutions of the cartilage extract were electrophoresed on 7.5% acrylamide gels containing 0.5 mg/ml of gelatin. The gels were washed two times with 2.5% Triton X-100 and incubated at 37°C for 18 h in 50 mM Tris/HCl, 10 mM $CaCl_2$, 1 μM $ZnCl_2$, 1% Triton X-100, 0.02% NaN_3, pH 7.5. The gels were then stained with 0.1% Coomassie blue in 40% methanol for 45 min. The gels were then destained in 7% acetic acid. Standards of recombinant MMP-2 and MMP-9 were included in the gel. The cleared gels were scanned in a UVP Image Capture system (UVP Inc., Upland, CA) and the area quantified for each band (both latent and active) relative to authentic standards of MMP-2 and MMP-9. The latent and active amounts of gelatinase were combined to give a total value for each gelatinase.

MMP INHIBITION ASSAY

The preparation of the human recombinant MMPs used in these studies has been described previously[32]. MMP inhibitors were tested for their ability to inhibit human MMPs using the quenched fluorescence assay[33]. This assay was modified to fit a 96-well format to increase the throughput. Assays for MMPs 1, 3, 8 and 13 employed human recombinant truncated enzymes. MMP-7 was assayed using recombinant catalytic domain. The optimal amount of each enzyme to produce significant and reproducible substrate cleavage was determined in preliminary experiments. Assays for MMP-2 and MMP-9 utilized human recombinant full length enzymes. MMP-2 was activated by incubating proMMP-2 with APMA (1 mM) for 45 min. The final concentration of MMP-2 in the assay mixture was 1 nM. ProMMP-9 was activated with MMP-3 (ratio 20:1) for 2 h and diluted to a final assay concentration of 0.75 nM. The final concentration of MMP-3 in the assay was 0.038 nM. The low concentration of MMP-3 in the final MMP-9 dilution did not contribute to the rate of substrate cleavage as assessed by control experiments with 0.038 nM MMP-3. MMPs 1, 3, 7, 8, and 13 were used at final concentrations of 8 nM, 16 nM, 2 nM, 4 nM and 0.5 nM, respectively. The MMP assays were performed using the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 at a concentration of 4 μM at 25°C. The assay buffer was 50 mM Tris, pH 7.5, 0.2 M NaCl, 5 mM $CaCl_2$ and 0.02% Brij-35. The increase in fluorescence due to cleavage of the substrate (Gly-Leu bond) was monitored kinetically for 30 minutes with a BMG Fluostar fluorescence plate reader (λ_{ex} 328 nm, λ_{em} 393 nm). Each 96-well microtiter plate contained 100 μl of substrate and 50 μl of enzyme in each well. 50 μl of MMP inhibitor was added to each well (except for positive control) to give a final volume of 200 μl/well. MMP inhibitors were tested at eight different concentrations and an IC50 was calculated using the formula: $V_i/V_o = 1/1+[I]/IC_{50}$ where V_i is the initial velocity of substrate cleavage in the presence of inhibitor at concentration [I] and V_o is the initial velocity in the absence of inhibitor.

Results

DOSE AND TIME DEPENDENCE OF RAT IODOACETATE-INDUCED ARTHRITIS

Rats were given a single intraarticular injection of various doses of iodoacetate into the knee joint and the severity of cartilage damage was monitored at 3 weeks, a time that preliminary experiments had determined was sufficient for development of cartilage damage. The severity of macroscopic cartilage lesions was proportional to the dose of iodoacetate injected [Fig. 2(A)]. Mild cartilage damage was observed at the low dose of 0.1 mg of iodoacetate (joint score 1.1±0.04 mean±S.E.M., N=10) whereas, severe damage involving the majority of the tibial plateau was observed at the high dose of 1 mg (joint score 3.9±0.04, mean±S.E.M., N=10). A dose of 0.25 mg was chosen for the time course studies since this dose resulted in moderate damage.

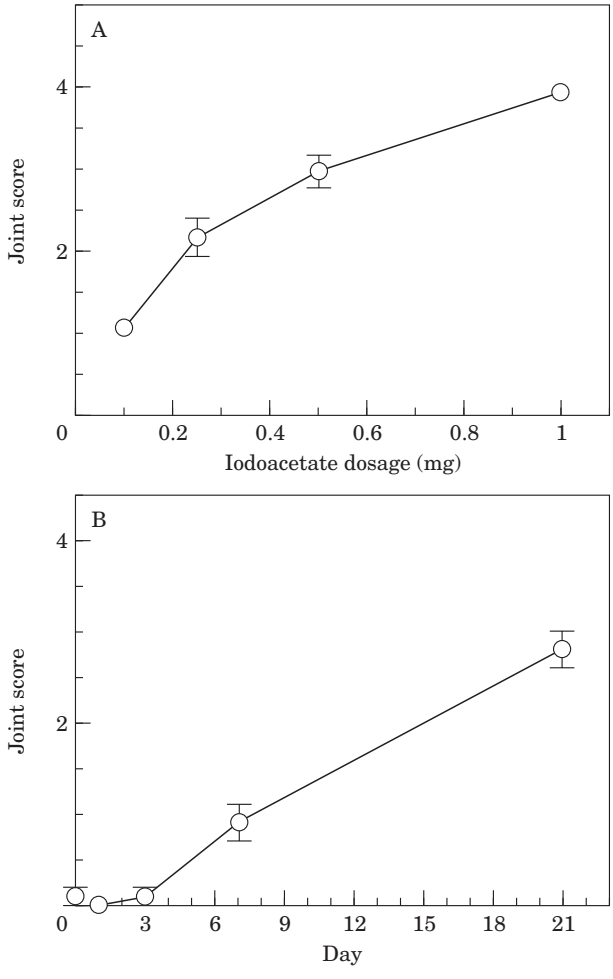


Fig. 2. Dose and time dependent effect of iodoacetate injection on the severity of damage in the knee joint of rats. The severity of cartilage damage in the knee of rats injected with varying amounts of iodoacetate (A) and assessed 21 days later and the effect of the injection of 0.25 mg of iodoacetate on the severity of cartilage damage (B) measured at varying times after injection. The data are presented as the mean±S.E.M. from 10 rats per group.

The injection of 0.25 mg of iodoacetate into the knees of rats resulted in a time related increase in the severity of cartilage damage [Fig. 2(B)]. Little macroscopic damage was observed at time points prior to one week [Fig. 2(B)]. Mild damage was observed 1 week after injection of iodoacetate (joint score 0.9±0.2, mean±S.E.M., N=10) and increased with time to a joint score of 2.8±0.2 (mean±S.E.M., N=10) at 3 weeks. Based on the dose response and time course studies, a dose of 0.25 mg of iodoacetate and 3 weeks duration was chosen to study the effect of MMP inhibitors. This time point and dose of iodoacetate provided sufficient cartilage damage allowing evaluation of the efficacy of compounds in a relatively short period of time thereby reducing the required quantity of inhibitor.

HISTOLOGICAL EVALUATION OF IODOACETATE-INDUCED ARTHRITIS

The evolution of arthritis after intraarticular injection of 0.25 mg of iodoacetate into the knee was evaluated histologically. The cartilage in the knees of rats that had received an intraarticular injection of 0.25 mg of iodoacetate 24 h prior to sacrifice showed a slight reduction in proteoglycan staining compared to untreated controls [Fig. 3(A),(B)]. By 72 h, the cartilage of the tibial plateau was characterized by a small central area of chondrocyte necrobiosis and reduced proteoglycan staining. The surrounding cartilage was swollen (i.e. its mean thickness had increased), and there was loss of proteoglycans from the superficial and middle zones [Fig. 3(C)].

At 1 week there was an extension of the area of central chondrocyte necrobiosis with cartilage thinning in the surrounding area, marked loss of proteoglycans and a decreased cell density [Fig. 3(D)]. At this time the first changes in the subchondral bone were appearing, characterized by resorption below areas of cartilage necrosis (data not shown). Three weeks after iodoacetate injection chondrocyte necrobiosis affected all of the cartilage with the exception of the very periphery of the articular surface of the tibial plateau. The cartilage was thin and there was no proteoglycan staining. There was some fibrillation of the cartilage [Fig. 3(E), inset]. Significant remodeling of the subchondral bone plate and calcified cartilage had occurred. This was manifest as subchondral bone sclerosis and deficiencies in the bone/calcified cartilage plate [Fig. 3(E),(F)]. These deficiencies contained either vascularized cores of fibrous tissue with infiltrating mononuclear cells or fibrocartilage. In some areas the fibrocartilage was more myxoid extending into marrow spaces where it was associated with osteoclastic bone resorption. These resembled the early subchondral bone cysts seen in human OA.

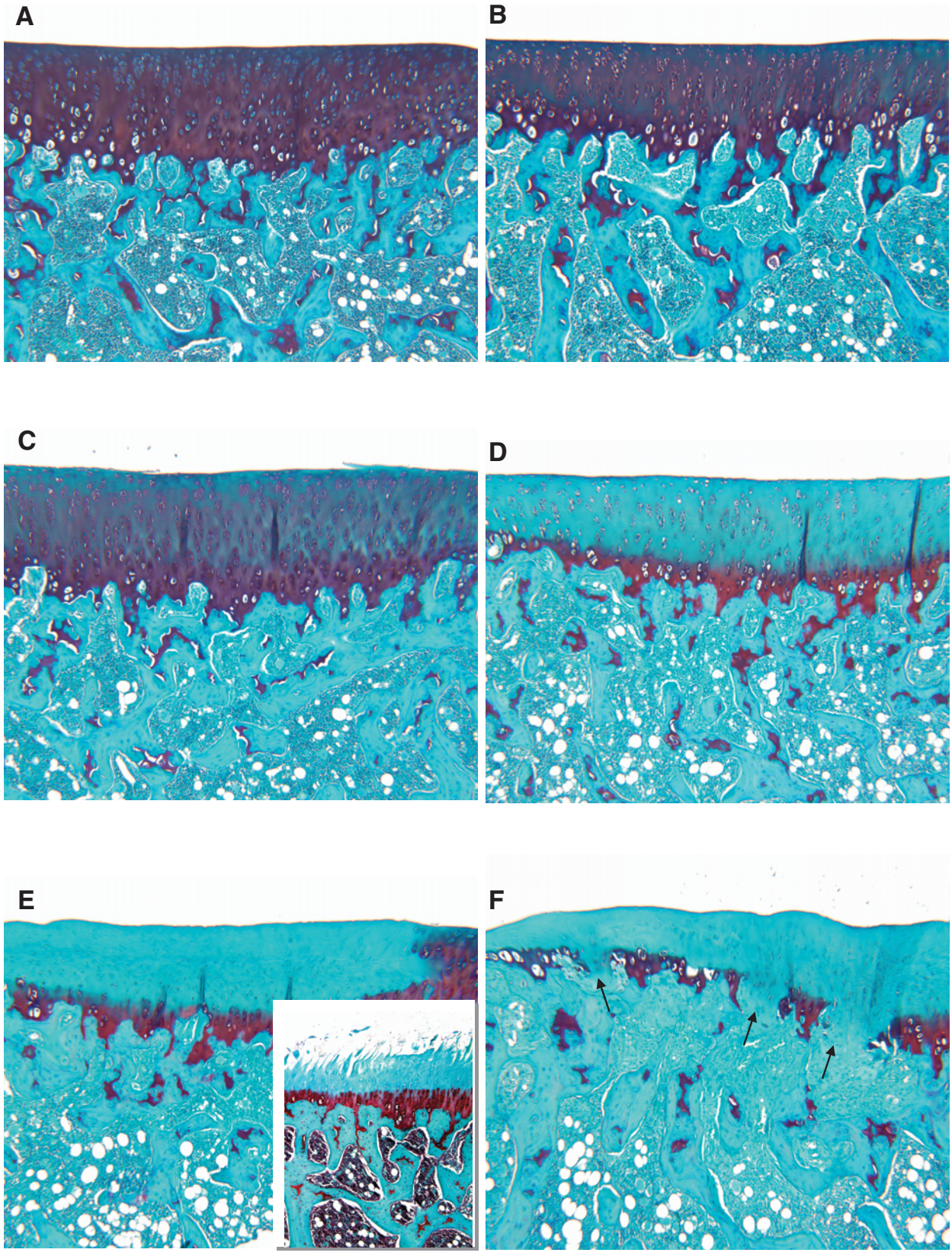
COLLAGENASE AND GELATINASE ACTIVITY IN JOINTS OF RATS INJECTED INTRAARTICULARLY WITH IODOACETATE

Rats were sacrificed at various times after intraarticular injection of 0.25 mg of iodoacetate and the knee joint that received iodoacetate was extracted as described in materials and methods. The extracts were evaluated for collagenase and gelatinase activity. There were detectable levels of collagenase and gelatinase activity in control rats that did not receive iodoacetate (Day 0) (Fig. 4). Both collagenase and gelatinase activity increased with time after iodoacetate injection up to day 7, after which there was a decline in activity (Fig. 4). The increase in collagenase activity in rats injected with iodoacetate was statistically significant at all time points tested with peak activity 4.1-fold above that of controls (Day 0) [Fig. 4(A)]. Gelatinase A (MMP-2) and gelatinase B (MMP-9) activity was significantly elevated on days 7 and 21 with peak activity at day 7 of more than three-fold above control values [Fig. 4(B),(C)].

INHIBITION OF IODOACETATE-INDUCED ARTHRITIS BY MATRIX METALLOPROTEINASE INHIBITORS

In vitro *inhibitory profile of the MMP inhibitors*

The MMP inhibitory profile of the compounds tested in the rat iodoacetate-induced arthritis model are shown in Table I. Compounds were evaluated for their ability to inhibit human recombinant MMPs 1, 2, 3, 7, 8, 9 and 13 in kinetic assays. Two of these compounds (PGE-6211443 and PGE-3162689) are broad spectrum inhibitors of human MMP's and one (PGE-6912923) is a potent inhibitor of the deep binding pocket MMPs but spares MMPs 1 and 7[22].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Although PGE-6912923s selectivity against MMP-1 cannot be evaluated in a rat model since this species lacks the MMP-1 gene, PGE-6912923 was included in these studies for its extremely potent inhibition of the deep pocket MMPs.

INHIBITION OF CARTILAGE DAMAGE BY MMP INHIBITORS (GROSS PATHOLOGY)

MMP inhibitors were administered orally to rats twice daily (35 mg/kg) during the first 7 days after iodoacetate administration and cartilage damage was assessed at day 21. All of the MMP inhibitors significantly decreased cartilage damage with inhibition ranging from 36–42% (Table II).

PGE 6912923 was evaluated for its ability to inhibit iodoacetate-induced arthritis at several doses. Administration of PGE 6912923 orally at 25, 12.5 and 5 mg/kg twice daily for 21 days resulted in a 35%, 20% and 20% inhibition of iodoacetate induced cartilage damage, respectively (Table III). Inhibition of joint damage was statistically significant (P<0.05) at all doses of PGE 6912923 tested (Table III).

INHIBITION OF JOINT DAMAGE BY MMP INHIBITORS (HISTOLOGY)

The effect of PGE 6912923 on iodoacetate-induced arthritis was evaluated histologically at day 21. Administration of PGE 6912923 orally at 25, 10 and 5 mg/kg twice daily for 21 days resulted in a 31%, 24% and 26% inhibition of the subchondral bone lesions, respectively. The inhibition of subchondral bone damage was statistically significant (P<0.05) at all doses of PGE 6912923 tested (Table III). Cartilage proteoglycan loss 3 weeks after iodoacetate injection was also recorded. The MMP inhibitors did not appear to protect the proteoglycan loss from cartilage as it was virtually complete in all treatment groups with scores of 3.92, 3.89, 3.96 and 4 (scale of 0–4) for the vehicle treated and PGE 6912923 treated (25, 10 and 5 mg/kg) groups, respectively.

Discussion

The matrix metalloproteinases have been implicated in the cartilage destruction that occurs in human[15,18,34] and animal models of OA[35,36]. The injection of iodoacetate into the knees of rats provides a model where lesions resembling some aspects of human OA can be quickly produced and has been suggested as a model for the study of chondroprotective drugs[8,30]. The rapid development of joint damage in the rat iodoacetate model has advantages over spontaneous or surgical models in larger animals as smaller amounts of inhibitor and shorter time periods are required. In the present study we have demonstrated that

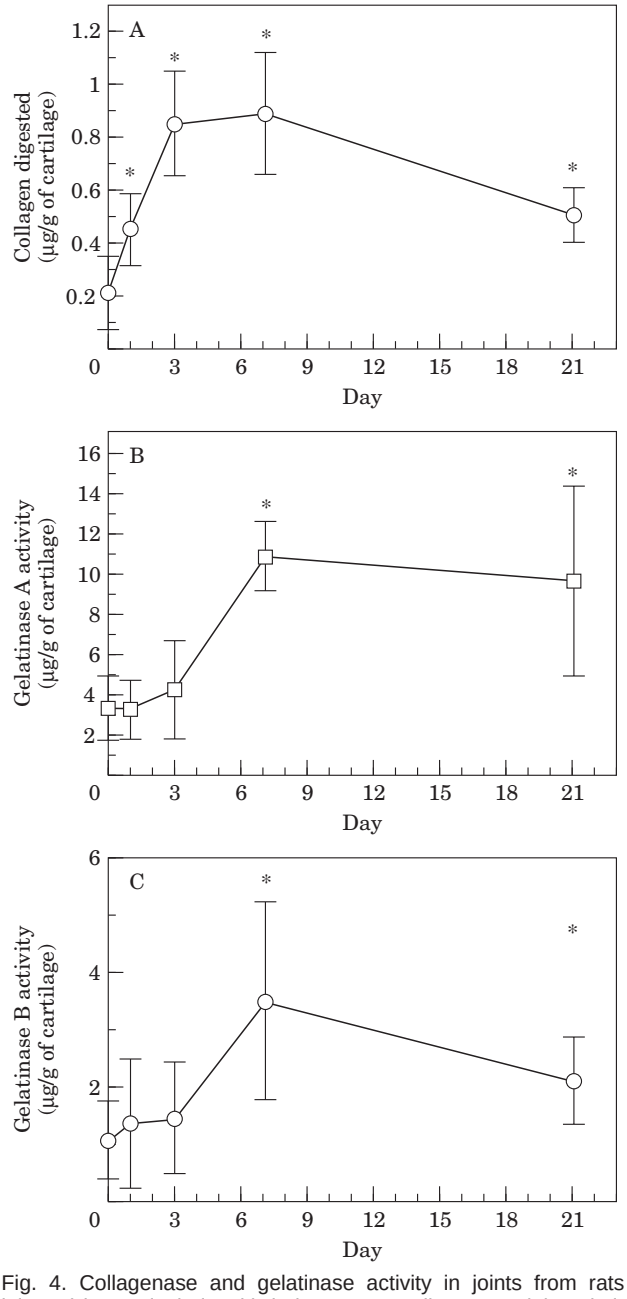

Fig. 4. Collagenase and gelatinase activity in joints from rats injected intraarticularly with iodoacetate. Collagenase (A), gelatinase A (B) and gelatinase B (C) were measured as described in materials and methods at various times after intraarticular injection of iodoacetate. The data are expressed as the mean ± S.D. from 5–6 rats (collagenase) and 5–7 rats for gelatinase A and B. *Denotes significance (P<0.05) from normal uninjected control animals using a two-sided Wilcoxon (non-parametric) test.

Fig. 3. Time course for the development of histological lesions in the rat iodoacetate model of arthritis. Coronal section of the medial tibial plateau: (A) 21 days after intraarticular injection of saline; (B) 1 day after injection of 0.25 mg of iodoacetate, showing a minor degree of proteoglycan loss; (C) 3 days after the injection of iodoacetate there is cartilage swelling and significant loss of proteoglycans from the superficial cartilage with horizontal fibrillation; (D) 1 week after injection of iodoacetate, marked loss of proteoglycan from all layers of the cartilage, cartilage thinning, and reduction of chondrocyte numbers in the superficial zone and focally in the mid zones; (E), (F) 3 weeks after injection of iodoacetate, the changes at this time are variable. The illustrations show the common appearances. There is widespread chondrocyte necrobiosis and loss of proteoglycans. Occasionally the appearance of the cartilage resembles fibrillation (inset). Cartilage thinning is sometimes extreme [left edge of (F)]. In places the calcified cartilage and subchondral bone plate are breached [(F) arrows] by cellular infiltrates and vascularized fibrous tissue. This is associated with bone sclerosis with woven bone formation and subchondral cyst formation. The sections were stained with Safranin O-fast green and the original magnification was ×100.

Table I
MMP inhibition profile for compounds evaluated in the rat iodoacetate model

Compound	IC_{50} (nM)						
	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13
PGE-6211443	13±7	3.7±1.1	7.7±2.3	153±37	1.4±0.8	2.9±1.4	1.9±1.1
PGE-3162689	4.3±0.7	1.2±0.2	19±2.5	2.1±0.1	0.7±0.2	1.8±0.6	1.4±0.3
PGE-6912923	143±19	<0.4	12±3	3894±665	<0.4	<0.4	<0.4

Data are the mean±S.D. from 3–4 experiments.

Table II
MMP inhibitors reduce cartilage damage in the rat iodoacetate model

Compound	Cartilage lesion score (mean±S.E.M.)	% reduction
Vehicle	2.48±0.17	—
PGE-6211443	1.6±0.18*	36
PGE-3162689	1.43±0.1*	42
PGE-6912923	1.47±0.14*	42

All compounds were dosed b.i.d. at 35 mg/kg for the first 7 days after rats received an intraarticular injection of iodoacetate. Animals were sacrificed 21 days after receiving iodoacetate, the left knee joint was disarticulated and the tibial plateau imaged and scored. The data are expressed as the mean cartilage damage score±S.E.M. from 15 animals per treatment group. *Denotes significance (P<0.05) from vehicle.

the injection of iodoacetate into the knees of rats resulted in an increase in MMP activity. In addition, MMP inhibitors were shown to significantly decrease the joint damage that occurs in this model.

Increased levels of collagenase and gelatinase enzymatic activity were observed in cartilage extracts after iodoacetate injection (Fig. 4). Collagenase and gelatinase activity increased during the first 7 days after iodoacetate injection with a decline in activity measured at day 21. We have previously reported that significantly elevated amounts of both MMP and aggrecanase proteoglycan cleavage sites were detected in cartilage of rats injected with iodoacetate by day 7 post-injection as detected using neoepitope antibodies specific to the C terminal epitopes DIPEN (MMP) and NITEGE (aggrecanase)[37]. In this study, MMP inhibitors were found to be efficacious in inhibiting cartilage damage when dosed exclusively during the first seven days of 21 day studies (Table II). These data suggest that MMPs and aggrecanase are important in the early stages of the evolution of cartilage damage in the rat iodoacetate model.

The maximum inhibition of cartilage damage after oral administration of an MMP inhibitor during the first seven days after iodoacetate injection was 42%. In studies with a number of different MMP inhibitors dosed for the entire 21 days after iodoacetate injection the maximum inhibition of cartilage damage observed was 52% (data not shown). These data are consistent with MMPs being important mediators of iodoacetate-induced cartilage damage but suggest alternative enzymes such as the cysteine proteinases cathepsin B[38] and K[39] or other mechanisms may also contribute.

The injection of iodoacetate induces the loss of cartilage proteoglycan as measured by safranin O-fast green staining (Fig. 3). The loss of cartilage proteoglycan staining has been reported in guinea pigs injected with a similar dose of iodoacetate (0.3 mg)[40,41]. Proteoglycan loss was followed by a severe thinning of the cartilage and the development of lesions in the region of the subchondral bone and calcified cartilage consisting of fibrous tissue, infiltrating mononuclear cells and blood vessels. Although areas of cartilage fibrillation were observed (Fig. 3), the subchondral/calcified cartilage lesion that is occasionally seen in human OA as areas of neovascularization, was a major histopathologic feature of this model at the 0.25 mg dose of iodoacetate at 3 weeks and was inhibited by the MMP inhibitors (Table III). The kinetics of the appearance of the subchondral lesions correlated with the development of the gross lesions with both becoming prominent by week 3 (Figs 2 and 3). The inhibition of the subchondral lesions as

Table III
Inhibitory effect of PGE-6912923 on joint damage in the rat iodoacetate model

Compound	Cartilage lesion score (mean±S.E.M.)	% reduction	Subchondral lesion score (mean±S.E.M.)	% reduction
Vehicle	2.37±1.9	—	2.58±0.16	—
PGE-6912923 25 mg/kg	1.53±0.14*	35	1.79±0.18*	31
PGE-6912923 12.5 mg/kg	1.9±0.22*	20	1.96±0.2*	24
PGE-6912923 5 mg/kg	1.9±0.16*	20	1.90±0.51*	26

All compounds were dosed b.i.d. for the duration of the 21-day study. Animals were sacrificed 21 days after receiving iodoacetate, the left knee joint was disarticulated and the tibial plateau imaged and scored. Histological sections were then prepared and scored as described in materials and methods. The data are expressed as the mean joint damage or histology lesion score±S.E.M. from 15 animals per treatment group. *Denotes significance (P<0.05) from vehicle.

assessed by histology correlated with the inhibition of the gross lesions as assessed by image analysis (Table III). In addition, the administration of an MMP inhibitor for the initial 7 days after iodoacetate injection was as efficacious as when given for the duration of the 21-day study (Tables II, III and unpublished observations). Therefore, we hypothesize that during the first 7 days after iodoacetate injection there is a rapid upregulation and release of metalloenzyme activity (Fig. 4) with matrix degradation in the cartilage and subchondral bone. The prominent subchondral lesions seen by histology and visualized grossly by image analysis through a greatly thinned and proteoglycan depleted matrix develop due to cellular infiltration into the subchondral region, possibly as an attempt at repair. MMP activity appeared to be associated with the development of the subchondral lesions as MMP inhibitors inhibited them. Therefore, the rat iodoacetate model provides a rapid method to assess potential MMP inhibitors *in vivo* in the organ of interest, the joint.

The loss of cartilage proteoglycan safranin O staining was observed after iodoacetate injection. None of the MMP inhibitors affected this loss of proteoglycan. There could be several reasons for the lack of proteoglycan protection by MMP inhibitors. The proteoglycan degradation may not be due to MMP activity. The degradation of proteoglycan in cartilage cultures stimulated with IL-1 has been shown to be mediated by an enzymatic activity known as 'aggrecanase' and not by MMPs[42–44]. Recently, in three different animal models of inflammatory arthritis the early loss of proteoglycan was associated primarily with aggrecanase cleavage in the interglobulin domain[45]. Therefore, the loss of proteoglycan early after the injection of iodoacetate may be due to aggrecanase and not MMP activity and this is not efficiently controlled by the MMP inhibitors. In fact, the MMP inhibitors used in the present study did not inhibit IL-1 stimulated glycosaminoglycan (GAG) loss from bovine nasal cartilage explants at concentrations as high as 25 μM (Janusz *et al*., unpublished data). In addition, these compounds were weak inhibitors of GAG release using aggrecanase containing supernatant prepared and assayed in a similar manner to that previously described by Arner *et al*.[46] (Janusz *et al*., unpublished data). Alternatively, the loss of the proteoglycan matrix could be due to reduction in the viability of the chondrocytes after iodoacetate injection. In this scenario an MMP inhibitor would not be expected to be of benefit.

In summary, previous studies have reported that iodoacetate injection into the knee of rats results in histopathological features of OA such as a loss of proteoglycan, cartilage degeneration, osteophyte formation, a dose-dependent reduction in spontaneous locomotion[30] and altered gait[47]. In the present study we have demonstrated the important role of MMPs in the joint destruction that occurs in the rat iodoacetate model of arthritis and the efficacy of MMP inhibitors. The inhibition of joint damage in this model by MMP inhibitors supports the further evaluation of the therapeutic potential of MMP inhibition in human OA.

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